⌘ *Author's Choice*

THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 287, NO. 25, pp. 21303–21315, June 15, 2012

Expression of Mitochondrial Non-coding RNAs (ncRNAs) Is Modulated by High Risk Human Papillomavirus (HPV) Oncogenes*[S]

Received for publication, November 23, 2011, and in revised form, April 23, 2012 Published, JBC Papers in Press, April 26, 2012, DOI 10.1074/jbc.M111.326694

Claudio Villota[‡§¶1], América Campos[‡§¶], Soledad Vidaurre[‡§¶], Luciana Oliveira-Cruz[‡§], Enrique Boccardo[‖], Verónica A. Burzio[‡§¶], Manuel Varas[‡§¶], Jaime Villegas[‡§¶], Luisa L. Villa[‖], Pablo D. T. Valenzuela[‡§], Miguel Socías[‡‡], Sally Roberts[§§], and Luis O. Burzio[‡§¶2]**

*From [‡]Andes Biotechnologies S.A., [§]Fundación Ciencia para la Vida, Zanartu 1482 7782272, and [¶]Facultad de Ciencias Biológicas, Universidad Andrés Bello, Republica 252, Santiago, 8370146, Chile, the [‖]Ludwig Institute for Cancer Research, São Paulo, CPO1323-903, Brazil, the **Departamento de Microbiologia, Instituto de Ciencias Biomédicas, Universidade de São Paulo CEP05508-900, Brazil , the [‡‡]Clinica Alemana, Santiago 7650567, Chile, and the [§§]Cancer Research UK Institute for Cancer Studies, University of Birmingham, Birmingham B15 2TT, United Kingdom*

Background: Antisense mitochondrial ncRNAs are down-regulated during oncogenesis by unknown mechanisms.
Results: High risk HPV E2 oncogene induces down-regulation of the antisense transcripts. Additionally, E6 and E7 induce expression of a new sense mitochondrial ncRNA.
Conclusion: HPV oncogenes modulate expression of mitochondrial ncRNAs.
Significance: During non-viral oncogenesis, cellular factor(s), analogously to E2, could induce down-regulation of the antisense mitochondrial ncRNAs.

The study of RNA and DNA oncogenic viruses has proved invaluable in the discovery of key cellular pathways that are rendered dysfunctional during cancer progression. An example is high risk human papillomavirus (HPV), the etiological agent of cervical cancer. The role of HPV oncogenes in cellular immortalization and transformation has been extensively investigated. We reported the differential expression of a family of human mitochondrial non-coding RNAs (ncRNAs) between normal and cancer cells. Normal cells express a sense mitochondrial ncRNA (SncmtRNA) that seems to be required for cell proliferation and two antisense transcripts (ASncmtRNAs). In contrast, the ASncmtRNAs are down-regulated in cancer cells. To shed some light on the mechanisms that trigger down-regulation of the ASncmtRNAs, we studied human keratinocytes (HFK) immortalized with HPV. Here we show that immortalization of HFK with HPV-16 or 18 causes down-regulation of the ASncmtRNAs and induces the expression of a new sense transcript named SncmtRNA-2. Transduction of HFK with both E6 and E7 is sufficient to induce expression of SncmtRNA-2. Moreover, E2 oncogene is involved in down-regulation of the ASncmtRNAs. Knockdown of E2 in immortalized cells reestablishes in a reversible manner the expression of the ASncmtRNAs, suggesting that endogenous cellular factors(s) could play functions analogous to E2 during non-HPV-induced oncogenesis.

Cancer is characterized by a dysregulation of cell cycle control mechanisms, resulting in uncontrolled cell growth. Oncogenes and tumor suppressors, when functioning together properly, regulate progression of normal cell proliferation. In cancer, however, mutations result in constitutive activation of oncogenes, inactivation of tumor suppressors, immortality, resistance to apoptosis, invasiveness, and metastasis (1). About 15–20% of cancers are associated with infection by DNA and RNA oncogenic viruses (2–4), and the study of these pathogens has been invaluable in the discovery of key cellular pathways that become dysfunctional during cancer progression. For example, oncoproteins E6 and E7 from high risk human papillomavirus (HPV)[3]-16 or -18 disable tumor suppressors p53 and Rb and up-regulate telomerase, fundamental changes for cell immortalization (5, 6). Interestingly, an apparently important step in the induction of cancer by oncogenic viruses is the specific interaction of some viral oncogenes with mitochondria, an organelle that has been implicated for decades in carcinogenesis (7, 8).

Human cells express a unique family of sense and antisense mitochondrial ncRNAs containing long inverted repeats (IRs) (9, 10). The sense transcript or SncmtRNA (hereafter referred to as SncmtRNA-1), which contains an 815-nt IR and consequently a stem-loop structure (9), is expressed in normal proliferating cells and tumor cells but not in resting cells. This correlation between cell proliferation and expression of the SncmtRNA-1 suggests a function for this transcript in cell cycle

* This work was supported by Fondecyt Grants 11090060, 1085210, and 1110835; Fondef Grant D04I1338; CCTE-PFB16 Program Grant; Conicyt, Chile, and Universidad Andrés Bello, Chile, Grants DI-34-09/R, DI-31-09/R, DI-28-09/R4, and DI-06-09/R.
⌘ *Author's Choice*—Final version full access.
[S] This article contains supplemental Figs. 1–6.
[1] To whom correspondence may be addressed: Andes Biotechnologies S.A., Zañartu 1482, Santiago, Chile. Tel.: 56-2-237-2066; Fax: 56-2-237-2259; E-mail: claudio.villota@gmail.com.
[2] To whom correspondence may be addressed. E-mail: lburzio@gmail.com.

[3] The abbreviations used are: HPV, human papillomavirus; ncRNA, non-coding RNA; SncmtRNA, sense non-coding mitochondrial RNA; ASncmtRNA, antisense non-coding mitochondrial RNA; HFK, human foreskin keratinocytes; ISH, *in situ* hybridization; ASO, antisense oligodeoxynucleotide; ASO-C, control ASO; IR, inverted repeat; nt, nucleotide(s); EdU, 5-ethynyl-2′-deoxyuridine; 16S mtrRNA, mitochondrial 16S ribosomal RNA; PML, promyelocytic leukemia.